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Indications of future performance of native and non-native adult oysters under acidification and warming

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ABSTRACT

Globally, non-native species (NNS) have been introduced and now often entirely replace native species in captive aquaculture; in part, a result of a perceived greater resilience of NSS to climate change and disease. Here, the effects of ocean acidification and warming on metabolic rate, feeding rate, and somatic growth was assessed using two co-occurring species of oysters – the introduced Pacific oyster *Magallana gigas* (formerly *Crassostrea gigas*), and native flat oyster *Ostrea edulis*. Biological responses to increased temperature and pCO_2 combinations were tested, the effects differing between species. Metabolic rates and energetic demands of both species were increased by warming but not by elevated pCO_2. While acidification and warming did not affect the clearance rate of *O. edulis*, *M. gigas* displayed a 40% decrease at 750 ppm pCO_2. Similarly, the condition index of *O. edulis* was unaffected, but that of *M. gigas* was negatively impacted by warming, likely due to increased energetic demands that were not compensated for by increased feeding. These findings suggest differing stress from anthropogenic CO_2 emissions between species and contrary to expectations, this was higher in introduced *M. gigas* than in the native *O. edulis*. If these laboratory findings hold true for populations in the wild, then continued CO_2 emissions can be expected to adversely affect the functioning and structure of *M. gigas* populations with significant ecological and economic repercussions, especially for aquaculture. Our findings strengthen arguments in favour of investment in *O. edulis* restoration in UK waters.

1. Introduction

Ocean acidification and warming (OAW) affects the behaviour, metabolism, and performance of a diversity of marine organisms (Barry et al., 2011; Kroeker et al., 2013). Early-life history stages, especially important in population persistence, are shown to be particularly vulnerable (Byrne and Przeslawski, 2013; Kurihara, 2008; Przeslawski et al., 2015). Additionally, calcifying species are especially at risk as they are susceptible to alterations in ocean chemistry (Hofmann et al., 2010; Parker et al., 2013; Pörtner et al., 2014), manifested by increased metabolism, respiration, and energy expenditure (Pörtner and Farrell, 2008). This is raising concerns for the continued provision of important ecosystem goods and services, particularly when considering the likely effects on the performance of biogenic calcifying species, often associated with the provision of such services (Lacoue-Labarthe et al., 2016; Lemasson et al., 2017; Sunday et al., 2016; Weatherdon et al., 2016).

Species most resilient to OAW may well be those best able to enhance their energy assimilation. A common way for marine organisms to balance their energy intake and expenditure is to increase their feeding rate (Ramajo et al., 2015; Sanders et al., 2013; Thomsen et al., 2012; Towle et al., 2015), or reallocate energy through partitioning and trade-offs between reproduction, somatic growth and calcification (Leung et al., 2017). Species less able to manipulate their feeding activity to offset stress from OAW may show reduced energetic levels and capacity for metabolic maintenance (Houlbrèque et al., 2015; Mackenzie et al., 2014; Vargas et al., 2015). OAW may therefore be an important selection pressure that dictates the distribution of species and functioning of marine ecosystems. Today, there is pressure to understand the effects of OAW on species that provide important ecosystem goods and services (Osborn et al., 2017) and mitigate negative impacts of OAW to ensure the sustainable delivery of the services derived from those species in to the future.

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In the UK, the native European flat oyster, *Ostrea edulis*, and the non-native Pacific oyster, *Magallana gigas* (which until recently was named *Crassostrea gigas*) are two valuable commercially-exploited species. They provide relatively similar and numerous ecosystem services (Herbert et al., 2012) including: reef formation, erosion control, improvement of water quality (through cycling and purification), raw material supply, and food provision (through aquaculture and fisheries) (see Coen et al., 2007, for a review of oyster-associated ecosystem services; Herbert et al., 2012). Historically, *O. edulis* was highly abundant and was the basis of a major shellfish fishery in the UK and Europe (Coolen, 2017; Orton, 1937), but today is a protected species in the UK with active restoration efforts underway to counteract ever declining stocks from overharvesting, competition, pests, diseases, and reproductive failures (Laing et al., 2006; Lallias et al., 2010; Woolmer et al., 2011). In contrast, *M. gigas* was introduced to the UK within regulated aquaculture settings in the mid-20th Century in response to the decline of *O. edulis,* and today this species represents over 90% of UK oyster aquaculture production, worth an estimated £10.14 million annually (Humphreys et al., 2014).

Magallana gigas was originally introduced under the assumption that local seawater temperatures would prevent its reproduction and the formation of viable wild populations, nonetheless the species has formed unintended wild populations on UK and Irish shores where it is often considered invasive (Dolmer et al., 2014; Herbert et al., 2016; Kochmann et al., 2013; Troost, 2010). Despite the occurrence of wild populations, the harvest of *M. gigas* is currently mostly limited to regulated aquaculture sites (Herbert et al., 2012). Today, beds comprised of both *M. gigas* and *O. edulis* occur, such as in Ireland (Zwerschke et al., 2017) and at sites along the South-West coast of the UK (pers. observations; Fig. 1a,b). It is often speculated that *M. gigas* and *O. edulis* compete for space and resources, with the presence of *M. gigas* having negative consequences for *O. edulis*, although there is no documented evidence of this. In fact, a recent study suggests no evidence of competition between the two species (Zwerschke et al., 2016). Nevertheless, the negative perception of wild *M. gigas* populations has led to management measures being introduced to prevent its further proliferation, and to promote the recovery of *O. edulis* (Harding et al., 2016; Herbert et al., 2012; Laing et al., 2006; Sawusdee, 2015; Woolmer et al., 2011).

Since its introduction to Europe, *M. gigas* has been spreading northward across European shores (Shelmerdine et al., 2017) facilitated by increasing average sea surface temperatures (SST) (Angles d'Auriac et al., 2017; Rinde et al., 2016; Thomas et al., 2016; Townhill et al., 2017). In contrast, the extent of *O. edulis* is continuing to decline, and native oyster reefs are considered some of the most endangered coastal habitats in Europe (Airoldi and Beck, 2007; Beck et al., 2011). The success of introduced species is often attributed to their greater tolerance (and physiological plasticity) to fluctuating environmental conditions than their native counterparts (Hall-Spencer and Allen, 2015; Lodge, 1993; Stachowicz et al., 2002). For example, in Australia, early-life stages of *M. gigas* (introduced) were shown to be less sensitive to OAW than the native *Saccrostrea glomerata* (Parker et al., 2010) and in Brazil, introduced *M. gigas* was more resilient to extreme hypercapnic conditions than the native *Crassostrea brasiliana* (Moreira et al., 2018). A similar response has also been shown in other taxa. For example, in Spain, the non-indigenous mussel *Xenstrobus securis* was found more resilient to reduced pH than the native *Mytilus galloprovinciallis* (Gestoso et al., 2016). This precedent would suggest it is not unreasonable to expect *M. gigas* to display similar tolerance in the UK, and be more resilient than its native counterpart *O. edulis* to future change in environmental conditions.

As calcifiers, both oyster species can be expected to be negatively impacted by ocean acidification (reviewed in Lemasson et al., 2017). The risks that ocean acidification pose to oysters were first highlighted in 2007 when hatcheries in the Pacific North-West region of the US suffered mass mortalities of Pacific oyster larvae. Upwelling of acidified water with low aragonite saturation (a principle biomineral used in shell maintenance) caused an 80% reduction in hatchery production and significant financial losses (Barton et al., 2015; Cooley et al., 2017). Since then, studies into the effects of OAW on oysters and other commercially important bivalves have rapidly increased in number. Extensive work has been done on early life stages, demonstrating sensitivity to OAW, but also other environmental stressors (Cole et al., 2016; Parker et al., 2017a). Responses include slower calcification (Waldbusser et al., 2016), delayed growth, and delayed or abnormal development (Gray et al., 2017; Parker et al., 2010; Waldbusser et al., 2015).

Less work has been undertaken on juveniles and adults, although impacts on early life stages has been shown to "carry-over" into these life-history stages (Hettinger et al., 2012, 2013b). Both juveniles and adults have shown altered immune response (Liu et al., 2016; Wang et al., 2016), reduced calcification and shell growth (Beniash et al., 2010; Waldbusser et al., 2011b; Wright et al., 2014), increased shell dissolution (Waldbusser et al., 2011a), and reductions in shell strength (Dickinson et al., 2012; Mackenzie et al., 2014; Welladsen et al., 2010). Crucial metabolic activities, such as respiration and feeding, can also be impacted (Comeau et al., 2008; Dove and Sammut, 2007; Scanes et al., 2017), the resulting stress likely leading to mortality and reduced population resilience, impaired biological functioning, and reduced ecosystem service provision (Lemasson et al., 2017).

Temperature is considered a major determinant of species and ecosystem structure and functioning, and can interact (often synergistically) with acidification (Harvey et al., 2013; see Lemasson et al., 2017 for a review on oysters). For *M. gigas*, its thermal range is reported as 1.8–35 °C (see FAO factsheet; Fig. 1a). While the thermal optima is not known for this species (and may vary as a result of local adaptation, see Sanford and Kelly, 2011), given its evolutionary origins, it is argued that in the UK, increasing average SST that is associated with climate change allows increased metabolic performance, individual growth, and range expansion. For *O. edulis*, the thermal range is less well defined and where data are available, the evidence is contradictory (Shelmerdine and Leslie, 2009). In one instance, temperatures higher than 20 °C have been shown to be suboptimal, negatively affecting growth, metabolism and filtration activity in juvenile *O. edulis* (Buxton et al., 1981), but conversely, cold has also been shown to limit larval production, recruitment, and growth below temperatures of 17.5 °C (Beiras et al., 1995; Davis and Calabrese, 1969; Orton, 1940; Robert et al., 2017; Walne, 1958). Differences in response may be related to dispersal capacity. *Magallana gigas* generate solely planktotrophic larvae, whereas *O. edulis* first brood (larviparous) before generating shorter planktonic duration planktotrophic larvae, which arguably limits dispersal capacity and promotes a greater likelihood of local adaptation (Bertness and Gaines, 1993) in *O. edulis* over *M. gigas* making developmental performance thresholds less clear.

It is therefore unclear how continued CO_2 emissions and associated increases in ocean acidification and warming will affect wild and harvested populations of *M. gigas* and *O. edulis* in the UK, nor what the consequences for ecological functioning and provisioning of ecosystem services will be. Substitution of one species for another, either partially or entirely, can produce significant ecological impacts (Krassoi et al., 2008), but since *M. gigas* is, in theory, able to provide similar ecological functions and ecosystem services as *O. edulis* (Herbert et al., 2016; Zwerschke et al., 2016) and is currently present in higher abundances, efforts to eradicate it may be unwise if it becomes increasingly prevalent under climate change.

In this study, we test the effects of OAW on the physiological responses of a native and a non-native species of UK oyster to assess changes in performance and determine the potential respective ecosystem service contribution of these species both today and in the future. Individual measures of performance were assessed using Standard Metabolic Rate (SMR), Clearance Rate (CR), and Condition Index (CI) under simulated warming and acidification scenarios over a 12 week

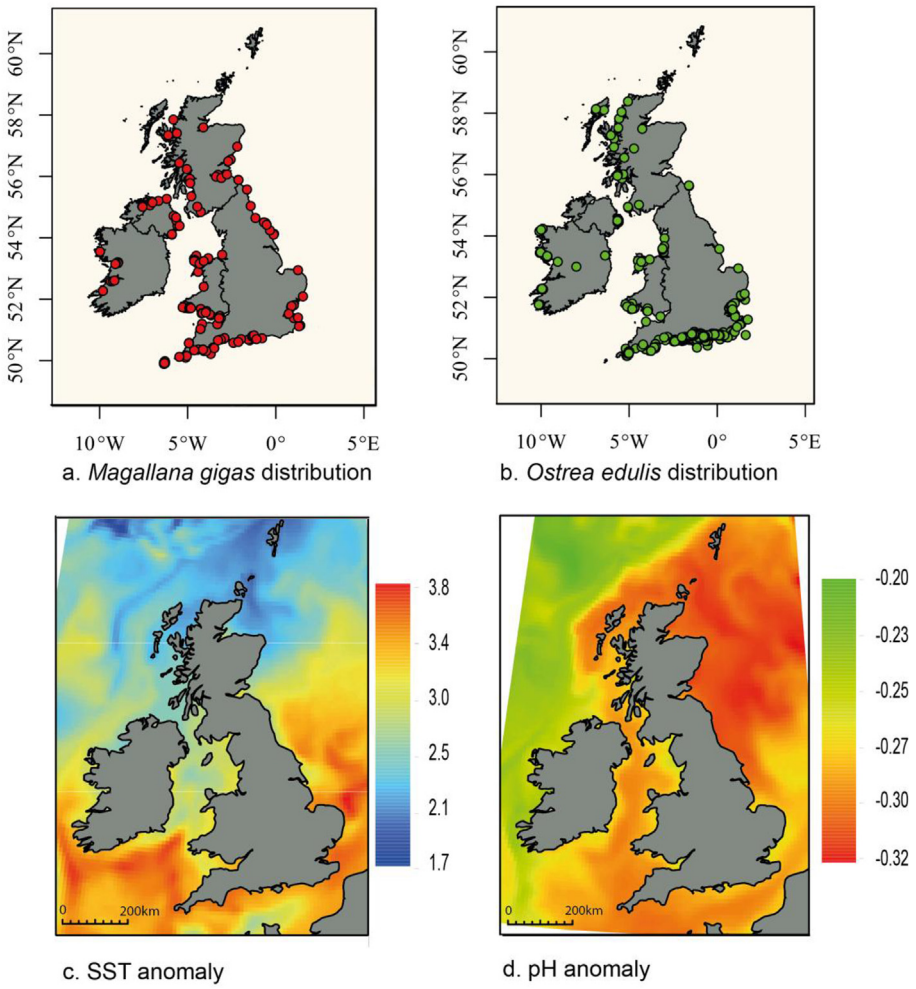

Fig. 1. Current UK wild distribution of (a) *Magallana gigas* (red) and (b) *Ostrea edulis* (green) (data obtained from the Global Biodiversity Information Facility (GBIF) database), (c) maximum mean annual sea surface temperature (SST) anomaly (SST, in °C; medium emission scenario IPCC SRES: A1B for 2070–2099, data obtained from UKCP09) and (d) minimum mean annual surface water pH anomaly (scenario for 2080–2099, data obtained from the Marine Ecosystem Evolution in a Changing Environment (MEECE) database). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

period. SMR was used as a proxy for metabolic costs and energetic requirements, while CR informed us of energy uptake. CI was used to assess overall health and quality and the availability of energy reserves within somatic tissues. Our hypotheses were that future OAW conditions would induce metabolic costs for both species of oysters, along with compensatory increases in energy acquisition through enhanced feeding. Additionally, we hypothesised that *M. gigas* would show evidence of higher tolerance to warming and acidification than *O. edulis*.

2. Methods

2.1. Organism collection and acclimation

Adult Pacific oysters (*M. gigas;* 112.4 ± 6.9 mm in length and weighing 285.9 ± 13.4 g), and European flat oysters (*O. edulis*; 79.4 ± 5.7 mm in length and weighing 92.8 ± 15.1 g) were hand-collected from a wild population at a low-intertidal fully marine site in Plymouth Sound, UK (50°23‘29.95“N, 004°13‘16.77“W), in July 2015 and January 2016, respectively. Oysters were cleaned of epibionts and allowed to acclimatise in a recirculating system to ambient laboratory conditions of ~16.5 °C and atmospheric pressure of 400 ppm at the University of Plymouth (UK). Over an acclimation period of 14 days, oysters were fed *ad libitum* with a mixed algal diet (Shellfish Diet, 1800; Reed Mariculture).

2.2. Experimental design

Following acclimation to laboratory conditions, 24 oysters were placed in their own 3 L experimental tank (four tanks per OAW scenario) and exposed to the treatment conditions. Three levels of pCO_2 (ambient 400 ppm, intermediate 750 ppm, elevated 1000 ppm), and two temperatures (control 16.8 °C, elevated 20 °C), were tested in an orthogonal experimental design to simulate current and future OAW scenarios. These six scenarios are in line with warming and acidification conditions predicted for the UK (Fig. 1c and d). As such, temperature scenarios reflected maximum current SST (16.8 °C), and predicted SST for the end of the century (20 °C, corresponding to the predicted increase by 3–4 °C in average SST along the South-West of the UK). However, it should be noted that such predictions do not taken into account localized variability in environmental conditions often experienced by organisms in coastal and estuarine habitats, and which may be amplified by future OAW. Due to capacity limitations of our mesocosm system, the experiment ran for 12 weeks between September and November 2015 for *M. gigas*, then repeated with *O. edulis* following the same procedures between January and March 2016. As such, the environmental conditions experienced by each species were inherently different due to natural seasonal variations in seawater properties driven by differences in atmospheric conditions (e.g. barometric pressure). The resulting pH conditions were therefore different between experiments (Fig. 2, S1 Table), but the effect size (magnitude of

difference in pH between experimental treatments) were comparable.

2.3. Mesocosm set-up

The ocean acidification and warming mesocosm system used during the experiment is a modified version of the one described by Calosi et al. (2013). Briefly, each treatment consisted of a header tank (volume = 80 L) of seawater, supplied from one of two sumps (16.5 °C and 20 °C), and aerated with either the ambient air pipe (pCO_2 400 ppm) or one of the two CO_2− enriched air pipes (pCO_2 750 ppm, pCO_2 1000 ppm). Ambient air consisted of laboratory air subjected to diurnal variability. Mixing in all header tanks was achieved using a submersible pump (Hydor Koralia Nano 900, Italy). CO_2 gas mix were obtained by slowly releasing CO_2 into two Buchner flasks where it mixed with ambient air, achieving two different levels of pCO_2, using multistage CO_2 regulators (EN ISO 7291; GCE, Worksop, UK). As such, throughout the experiment the three CO_2 levels varied in a similar manner following natural variations in CO_2 in the ambient air. The treatments thus took account of natural daily variability, which has been suggested as a critical consideration for climate change experimental studies (Humphreys, 2016; Reum et al., 2015). CO_2 levels in the two CO_2-enriched pipes were recorded using a CO_2 analyser (LI-820; LI-COR, Lincoln, NE, USA) and adjusted manually to the desired level twice daily. CO_2 levels in the ambient air pipe were also recorded to monitor the levels of the control treatments. Seawater was gravity-fed from the header tanks to each of the corresponding replicate tanks (3 L transparent sealed containers) at a constant rate of ~60 mL/min. The replicate tanks were held within four larger 300 L holding trays, each sump supplying seawater to two of the holding trays, effectively creating water baths maintaining the replicate tanks at the desired temperature (two water baths at 16.5 °C, two water baths at 20 °C). Each tray held two replicates of each CO_2 levels (four replicates per temperature and CO_2 treatment). Excess seawater was allowed to overflow from the trays to their corresponding sump, where it was filtered, aerated, and recirculated to the corresponding header tanks and trays using a submersible pump (1262; EHEIM GmbH and Co. KG, Deizisau, Germany). Seawater in the system originated from Plymouth Sound (UK) and, following mechanical filtering and UV sterilization, was added and replaced on a daily basis to account for evaporation, Deionized water was added as needed to maintain stable salinity levels. In elevated temperature treatments, seawater was increased to 20 °C using aquarium heaters (50 W aquarium heater; EHEIM Jager GmbH and Co. KG, Stuttgart, Germany) placed in header tanks and holding trays.

2.4. Measurements of seawater parameters

Temperature, salinity, and pH were measured daily in all replicate tanks (Fig. 2 see also S1 Table and S1 Fig. for details of temperature and pH data). Salinity was measured using a handheld refractometer (D&D The Aquarium Solution Ltd, Ilford, UK) and temperature measured using a digital thermometer (TL; Fisher Scientific, Loughborough, UK). pH was measured using a microelectrode (InLab® Expert Pro-ISM; Mettler- Toledo Ltd, Beaumont Leys, UK) coupled to a pH meter (S400 SevenExcellence™; Mettler-Toledo Ltd, Beaumont Leys, UK), following calibration with NIST traceable buffers. pH in the header tanks was also monitored (data not shown). Total Alkalinity (A_T) was measured once a week in each of the replicate tanks. 125 mL water samples were transferred to borosilicate bottle with Teflon caps and poisoned with 30 μL of saturated $HgCl_2$ solution (0.02% sample volume) before being kept in the dark until measurement by automatic Gran titration (Titralab AT1000 © Hach Company). Partial pressure of carbon dioxide (pCO_2) and saturation states of calcite and aragonite (Ωcalcite and Ωaragonite), were calculated at the end of the experiment using CO2 SYS (Pierrot et al., 2006), employing constants from Mehrbach et al. (1973) refitted to the NBS pH scale by Dickson and Millero (1987) and the KSO4 dissociation constant from Dickson (1990) (Fig. 2. see also S1 Table).

Throughout the duration of the experiment, oysters were fed daily with 20 mL of a live algae (mixed diet of *Isochrysis galbana* and *Tetraselmis* sp.) to obtain a concentration of approximately 10^8 cell.L^{-1} within the experimental tank. Three times a week, tanks were gently brushed and siphoned to remove faeces and excess food, thereby insuring acceptable water quality, removing no more than 20% of the volume, and left to slowly refill with the incoming equilibrated seawater.

2.5. Physiological measurements

Following 10 days, 5, 9, and 12 weeks of exposure to each OAW scenario, metabolic activity and energy acquisition were measured for each oyster (N = 24 per species; 4 per OAW scenario). To limit post-prandial metabolism of food and excretion of faeces that could alter the results, oysters were not fed for 24 h prior to measurements.

2.5.1. Standard metabolic rate

Respiration rates were measured as proxy for Standard Metabolic Rates (SMR), using microfiber optic oxygen sensors (Firebox 4, PreSens Germany, www.presens.de). All oysters (N = 24 per species; 4 per OAW scenario) was placed in a 1.2 L air-tight container, filled with 1 L of seawater filtered to 2 μm and pre-equilibrated to their respective experimental pCO_2 and temperature treatment. To maintain stable temperature in the chambers, all measurements were conducted in controlled-temperature rooms. The seawater in each chamber was stirred using a magnetic rod for the duration of the assay (350 rpm). Respiration measurements started when the oyster resumed filtration, and ended either when O_2 saturation reached 80% to prevent the organisms from experiencing hypoxic conditions, or when the oyster shut its valves. O_2 measurements were corrected for temperature, salinity, and barometric pressure using Green and Carritt's (1967) oxygen solubility coefficients and Weiss (1970) vapour pressure values, as well as corrected for background bacterial respiration (the reduction in dissolved oxygen in each tank without shellfish was subtracted from total O_2 reductions in the same tank with shellfish) and the individuals' volume and dry weight, to obtain absolute quantities of oxygen consumed. Temperature and salinity was recorded at the start of each assay as described above. Barometric pressure data were obtained from the Plymouth Live Weather Station (http://www.bearsbythesea.co.uk). Dry weight was assessed at the end of the 12-wk exposure (see below "Condition Index" section for details). Volume was determined using the water displacement method. SMR was calculated as follows:

$$SMR = \frac{V_r(L) \times \Delta C_w O_2 (mgO_2.\ L^{-1})}{\Delta t\,(h) \times bw\,(g)} \quad (1)$$

where SMR is the oxygen consumption normalized to 1 g of dry tissue mass (DW) in $mgO_2.g^{-1}$ $DW.h^{-1}$; V_r is the volume of the respirometry chamber minus the volume of the oyster (L); $\Delta C_w O_2$ is the change in water oxygen concentration measured (mgO_2 $.L^{-1}$); Δt is measuring time (h); and *bw* is the dry tissue mass (g) of the oyster.

2.5.2. Clearance rates

Directly following the respirometry assay, the Clearance Rate (CR) of all oysters from each treatment (n = 4) was calculated using methods previously described in Coughlan (1969) and Sanders et al. (2013). Individuals selected for clearance rate measurements were the same individuals used for the respirometry assay described above. Oysters were placed in a 1.2 L chamber, filled with 1 L of seawater filtered to 2 μm and pre-equilibrated at their respective experimental pCO_2 and temperature treatment. To maintain stable temperature in the chambers, all measurements were conducted in controlled-temperature rooms. ~20 mL of the same live algae culture (mix of *Tetraselmis* sp.

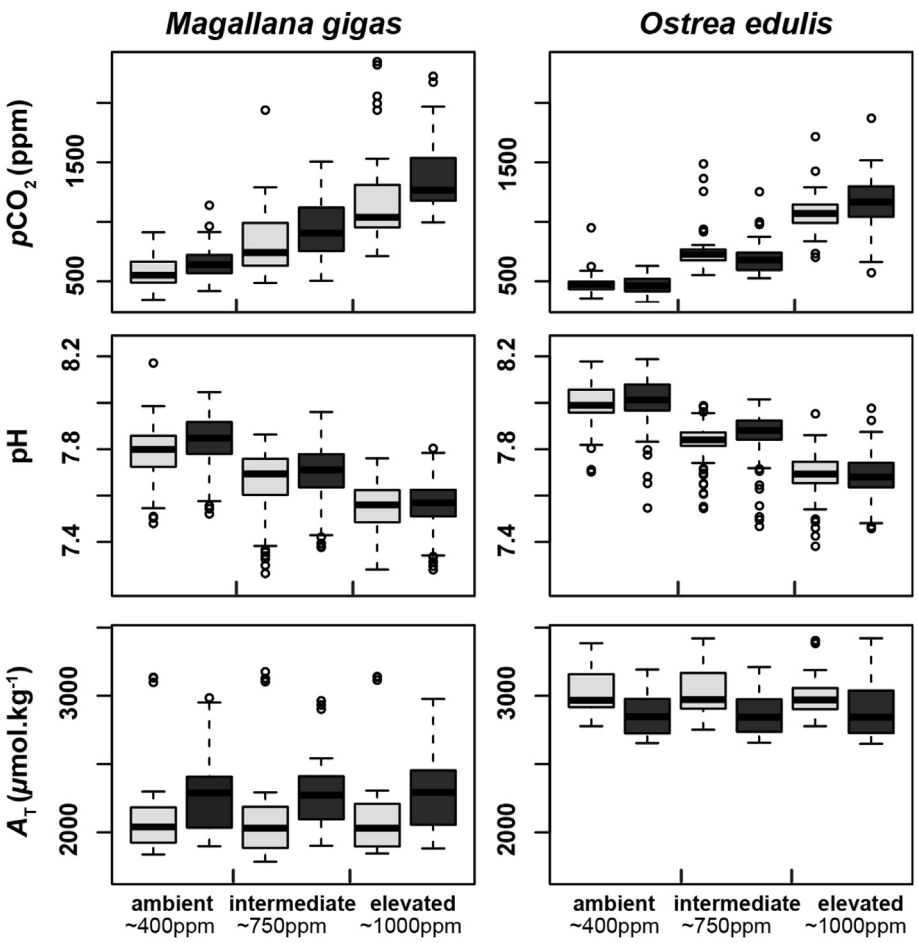

Fig. 2. Variation in pCO_2, pH, and total alkalinity (A_T), of seawater in the experimental treatments. ppm = part per million. Grey = control temperature (~16.8 °C, black = elevated temperature (~20.0 °C). Data are pooled based on daily (pH) and weekly (A_T) measurements over the 12 week experimental duration. Weekly pCO_2 values were calculated using CO2 SYS (Pierrot et al., 2006).

and *Isochrysis galbana*) was added to each chamber when oysters started filtering. To allow homogeneous mixing of algae, the seawater in each chamber was stirred using a magnetic rod (350 rpm). Three replicate 5 mL water samples were taken from haphazard locations throughout the chamber (1) prior to the addition of food (t_i); (2) immediately after addition of food (t_0) to check the initial algal concentration; and (3) at 10 min intervals following food addition for a duration of 40 min, providing 6 sampling times (i.e. t_i, t_o, t_1, t_2, t_3, and t_4). If the oyster shut its valves, the chronometer was stopped and restarted once the valves re-opened. Counts of algae in all water samples were performed in triplicate using a Coulter Counter (Beckman Coulter Z2). Clearance rates (CR) were calculated using the following equation after Coughlan (1969):

$$CR = \frac{V \times \ln\left(\frac{C_{n-1}}{C_n}\right)}{t_n - t_{n-1}} \quad (2)$$

where CR is the clearance rate measured during the 10 min interval between sampling times t_{n-1} and t_n, normalized to 1 g of dry tissue mass ($L^{-1}.g^{-1}DW.h^{-1}$), V is the volume of the chamber in L, C_{n-1} is the concentration (cell.L^{-1}) in the sample taken at time t_{n-1} (hour), and C_n is the concentration (cell.L^{-1}) in the sample taken at time t_n (hour). Results are presented as CRmax, the maximum clearance rate observed during the 40-min incubation.

2.6. Condition index

The Condition Index (CI) of oysters was calculated at the end of each experiment based on dry weight following the method recommended by Lucas and Beninger (1985) and described in equation (3). Condition indices are useful tools widely used in the aquaculture sector to evaluate the overall quality and health of bivalves (Knights, 2012; Marin et al., 2003). They reflect their ability to withstand adverse conditions (Marin et al., 2003) by describing the quantity of organic tissue present (Bodoy et al., 1986).

$$CI = \frac{dry\ meat\ weight}{dry\ shell\ weight} \times 100 \quad (3)$$

Dry tissue weight was determined after each oyster was shucked using an oyster knife and oven-dried at 105 °C until a constant mass was achieved.

2.7. Statistical analyses

All data were tested for the assumption of homogeneity of variances, and where not met, data were transformed using logarithmic or square-root transformations. If after transformations assumptions were still not met, equivalent non-parametric tests were conducted. Differences were considered statistically significant if $p < 0.05$. All data were analysed using the public domain software *R* (version 3.2.5 R Core Team, 2016). Due to natural variations in the chemistry of the seawater used during the experiments and the partial pressure of ambient air used, the treatments applied to each species were not consistent, and therefore, species were not formally compared and data analysed separately.

2.7.1. SMR and CR

SMR and CR data were analysed using linear mixed effects (lme) models with an autocorrelation argument (nlme package; see Zuur et al. (2009)). 'Temperature' and 'pCO_2' were considered as fixed factors to assess differences in species' response to the treatments, and 'Exposure'

(levels: 10 days, 5 wk, 9 wk, 12 wk) nested within 'Replicate' to partition differences due to individual oysters. If significant differences were present, *post-hoc* test was performed to assess differences between treatment levels (TukeyC and Multicomp packages). For each species, data were interrogated for the presence of fundamental relationships between the two physiological traits using the Pearson's correlation test.

2.7.2. Condition index

Differences in CI with treatment were analysed using 2-factor ANOVA with 'temperature' (levels: 'control'; 'elevated') and 'pCO_2' (levels: 'ambient 400 ppm', 'intermediate 750 ppm', 'elevated 1000 ppm') as fixed factors. If significant differences were present, *post-hoc* pairwise comparisons (Tukey HSD) were performed to determine differences between treatment levels.

3. Results

3.1. Standard metabolic rate

For both species, there was clear inter-individual variability in responses (Fig. 3.). The linear mixed-effects model revealed differences in metabolic response depending on exposure and OAW scenario. For *M. gigas*, higher temperature, but not pCO_2, increased SMR by > 43% (Fig. 3. $F_{1,18} = 11.51$, $p < 0.01$). For *O. edulis*, exposure time led to a statistically significant decrease in SMR after 5 weeks ($F_{1,71} = 25.55$, $p < 0.001$), and temperature led to a statistically significant increase in SMR of > 39% ($F_{1,18} = 9.52$, $p < 0.01$). However, it should be noted that for *O. edulis,* while the interaction between temperature and pCO_2 was marginally not significant ($F_{1,66} = 3.50$, $p = 0.052$), clear trends were apparent. SMR decreased by up to 36% under elevated pCO_2 conditions (750 and 1000 ppm) when oysters were kept at the control temperature, but when the temperature was elevated, there was no change in SMR even when pCO_2 was increased. This was especially notable under 1000 ppm pCO_2, where SMR was ~46% lower in the control temperature than in the warm temperature treatment.

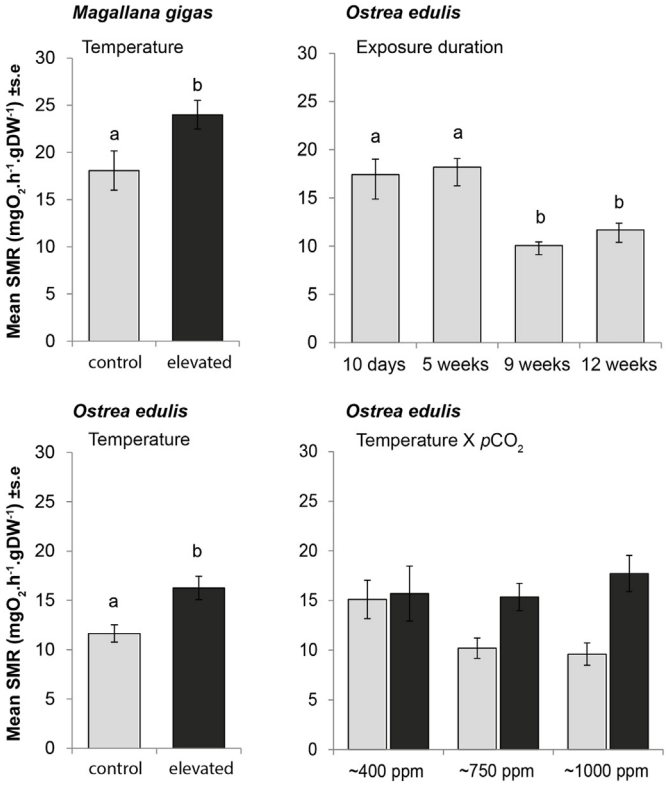

Fig. 3. Changes in standard metabolic rates (SMR) of *M. gigas* (top left) and *O. edulis* (bottom left) with temperature treatment; and of *O. edulis* with exposure duration (top right) and the interaction of temperature and pCO_2 treatments (bottom right). Grey = control temperature. Black = elevated temperature. DW = dry weight. Treatment groups that do not share a letter are significantly different.

3.2. Clearance rate

Again, for both species, there was clear inter-individual variability in responses. The linear mixed-effects model revealed differences in feeding response depending on exposure and OAW scenario. For *M. gigas*, pCO_2 ($F_{2,18} = 5.8$, $p < 0.05$) and exposure time ($F_{1,66} = 11.3$, $p < 0.001$) had significant effects on CRmax (Fig. 4.). Intermediate pCO_2 (750 ppm) led to ~40% decrease in CRmax in comparison to ambient pCO_2 conditions (Fig. 4 top left). While not statistically significant, there was evidence that suggests an interaction between temperature and pCO_2 on CRmax (Fig. 4 top right). Under control temperature, CRmax was 1.1 ± 0.2 $L\,h^{-1}.gDW^{-1}$ at ambient pCO_2 but when oysters were exposed to elevated pCO_2, CRmax either decreased by ~41% (750 ppm) or increased by ~45% (1000 ppm). Elevating the temperature led to an increase in CRmax (~91%) under ambient pCO_2; an effect that was then lost under the 750 ppm and 1000 ppm OA treatments, with CRmax returning to a level comparable with this species held under control temperature and ambient pCO_2 conditions (Fig. 4 top right). After 12 wk, CRmax had decreased by ~41% of the starting clearance rate (Fig. 4 bottom left).

For *O. edulis*, CRmax was affected by a combination of temperature and exposure time, but not pCO_2 ($F_{1,70} = 11.2$, $p < 0.001$) (Fig. 4 bottom right). Under control temperature, CRmax was not different at 10d, 9 and 12 wk, although there was a reduction in CRmax of ~41% at wk-5. Under elevated temperature, CRmax of *O. edulis* was 2.9 ± 0.5 $L\,h^{-1}.gDW^{-1}$ after 10d exposure (an increase of ~67% over control temperature oysters), but which subsequently dropped back to a rate comparable to oysters reared under control temperature for the remainder of the study.

3.3. Relationship between the physiological traits

There was no correlation between SMR and CRmax for either *M. gigas* or *O. edulis*.

3.4. Condition index

At the end of the exposure duration, none of the oysters were reproductive. For *M. gigas*, the effects of temperature and pCO_2 were only marginally not significant most likely due to statistical power ($F_{2,18} = 3.46$, $p = 0.053$), but clear trends were apparent. Under ambient temperature, there was no change in mean CI irrespective of pCO_2, but when temperature was elevated, there was a sustained reduction in mean CI with increasing pCO_2 (Fig. 5(centre)). Considering temperature or pH alone, temperature led to a 40% reduction in CI from 3.5 ± 0.2 to 2.1 ± 0.2 ($F_{1,18} = 12.5$, $p < 0.01$, Fig. 5(left)) but pCO_2 had no effect ($F_{2,18} = 0.56$, $p = 0.58$). In *O. edulis*, neither temperature ($F_{1,17} = 0.85$, $p = 0.37$) or pCO_2 ($F_{1,17} = 0.10$, $p = 0.902$) had any effect on CI, which averaged at 2.6 ± 0.1 (Fig. 5(right)).

4. Discussion

Climate change represents an important selection pressure dictating the distribution of species and the functioning of marine ecosystems. Today, there is pressure to understand the effects of multiple stressors on species that provide important ecosystem goods and services (Osborn et al., 2017), and to mitigate any negative impacts in order to ensure the sustainable delivery of these goods and services. Here, following exposure to temperature and pCO_2 scenarios predicted for the

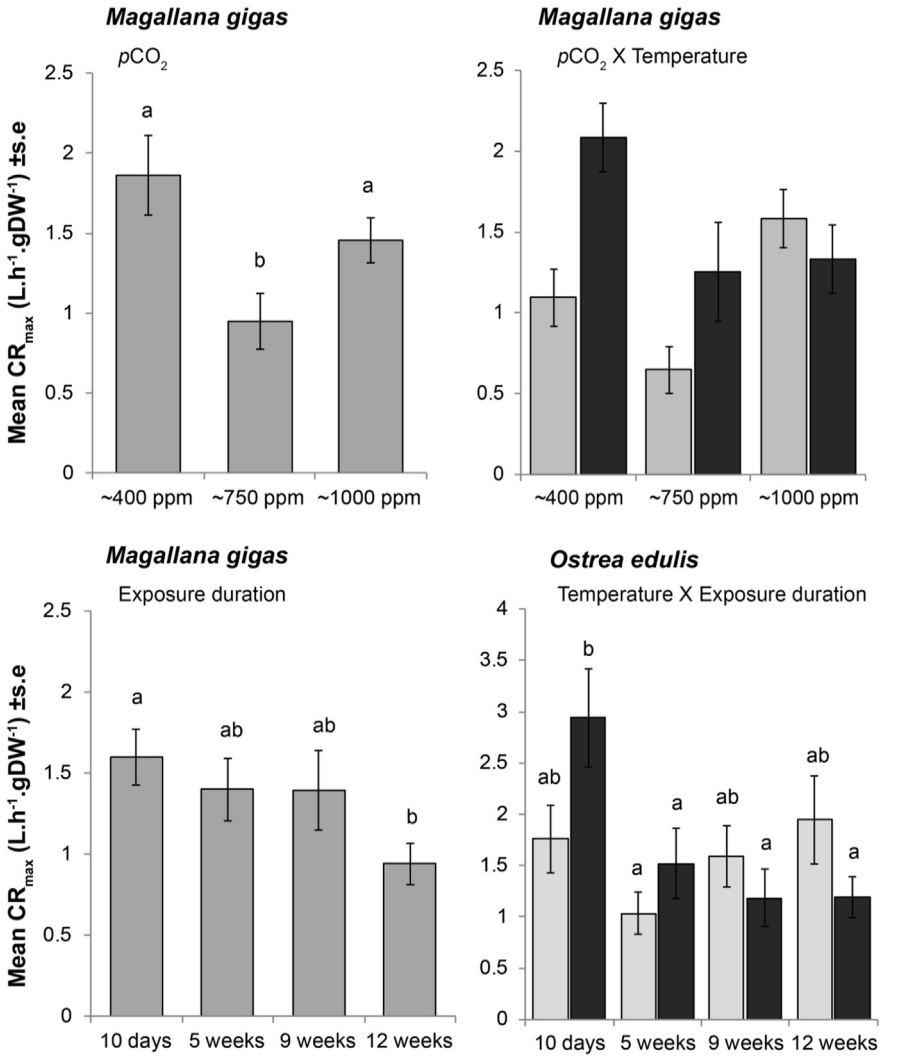

Fig. 4. Changes in maximum clearance rate (CRmax) of: *M. gigas* with pCO_2 treatment (top left), pCO_2 and temperature (top right), exposure duration (bottom left); and *O. edulis* with exposure duration (bottom right). Grey = control temperature. Black = elevated temperature. Treatments that do not share a letter are significantly different. DW = dry weight.

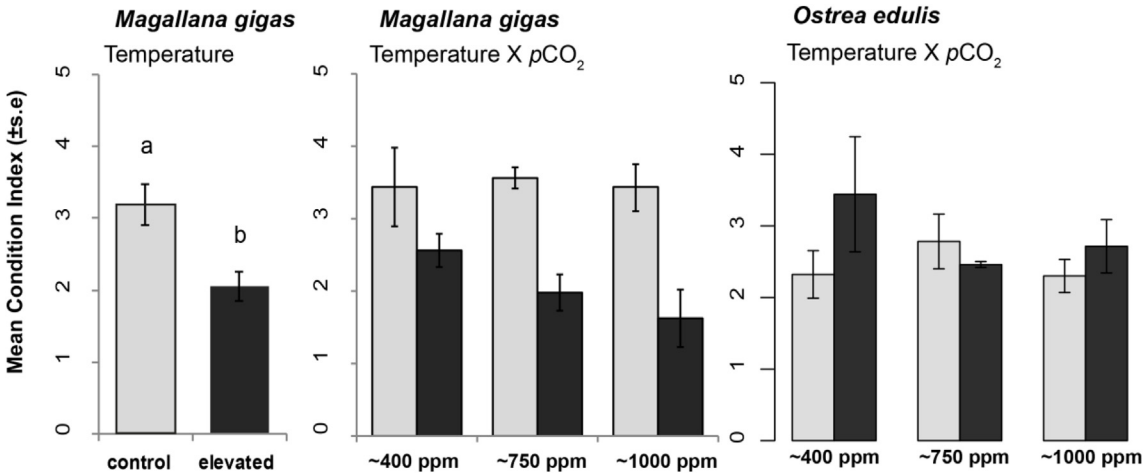

Fig. 5. Variations in the condition index of *M. gigas* and *O. edulis* across temperature and pCO_2 treatments after 12 weeks exposure. Grey = control temperature. Black = elevated temperature. *M. gigas*: n = 4; *O. edulis*: n = 4.

near future, we show species-specific changes in metabolic rate, feeding rate, and condition of two ecologically and economically important species of oysters. Contrary to expectations, non-native *M. gigas* experienced more pronounced negative effects of warming and acidification than the native *O. edulis*, displaying increased metabolic rate under elevated temperature to 20 °C, but decreased feeding rate under 750 ppm pCO_2, which led to reduced overall condition after 12 weeks. *O. edulis* appeared relatively unimpacted by future OAW scenarios.

4.1. Metabolism

In marine organisms, the performance of routine activities such as growth, reproduction, and feeding is supported by the metabolism of oxygen, which is modulated by environmental conditions such as temperature (Pörtner and Farrell, 2008). Throughout our experiment, the metabolic rate of *M. gigas* was affected by elevated temperature only. Overall, a ~3 °C temperature increase led to a > 43% increase in the SMR of *M. gigas*. Similarly, the metabolism of *O. edulis* also increased with elevated temperature by ~39%, although unlike *M. gigas*, this increase coincided with highest pCO_2 (1000 ppm) concentrations. This suggests that both *Magallana gigas* and *Ostrea edulis* display some capacity to withstand ocean acidification and warming scenarios in the short term, but elevated temperatures may pose a threat to functioning should increases in metabolism approach maxima.

Temperature increasing the metabolism of organisms is common in ectotherms; an effect previously shown in oysters (Bougrier et al., 1998; Saucedo et al., 2004; Shpigel et al., 1992) and other bivalves (Artigaud et al., 2014; Matoo et al., 2013). This is not necessarily problematic if temperature elevations are within the thermal window of the organism, but ocean warming is expected to push species closer to or beyond their upper thermal limit with physiological and ecological consequences. This is especially true for individuals already living close to their upper thermal limit (Pörtner and Farrell, 2008). In the UK, *M. gigas* is considered to be living in the middle of its thermal range; its capacity to increase metabolic rate under elevated temperature supports this assertion. The thermal limits of *O. edulis* are less well known, but here, individuals were able to increase metabolic rate under elevated temperatures, suggesting some biological scope to withstand the climate scenarios predicted for the future.

In our study, adult *M. gigas* and *O. edulis* displayed complex responses to variations in pCO_2 conditions, although none significantly changed their SMR, indicating that acidification levels tested here (750 ppm; 1000 ppm pCO_2) might not constitute stressful conditions for them. It is likely that these levels are not unusual in coastal and estuarine waters, and organisms may well have been subjected to these pCO_2 levels before (Hales et al., 2016). However, the metabolic response of bivalves to elevated pCO_2 appears species and population-specific. Several other studies examining the effect of pCO_2 on respiration rate in bivalves at concentrations equivalent to those tested here also revealed no change in SMR (e.g. *Crassostrea virginica* (at 800 ppm - Matoo et al., 2013), Mediterranean mussels *Mytillus galloprovinciallis* (at ~1090 ppm - Gazeau et al., 2014), and scallops, *Pecten maximus* (at either 750 ppm and 1140 ppm - Sanders et al., 2013)). Pronounced changes in respiration rates can be shown when pCO_2 levels greatly exceed those tested here (e.g. increasing in *C. virginica* (at 3500 ppm - Beniash et al., 2010) and *Mytilus edulis* (at 1120 ppm and 2400 ppm - Thomsen and Melzner, 2010), but reducing in *Ruditapes decussatus* (between 1698 ppm and 4345 ppm - Fernández-Reiriz et al., 2011)). It is argued that increases in metabolic rates allow individuals to maintain their internal acid-base balance and mainain routine physiological activities, such as biomineralization (Melzner et al., 2009; Pörtner and Farrell, 2008) although the conditions used to stimulate these changes greatly exceed pCO_2 concentrations predicted for the next 80 years.

Previously, interactive effects of pCO_2 and temperature on metabolism have been shown (e.g. Lannig et al., 2010, at ~1480 ppm and 20 °C or 25 °C); an effect reinforced in *O. edulis* in this study which showed that elevated temperature could compensate for the decreasing trend in SMR under elevated pCO_2 (1000 ppm) and lead to an overall increase in SMR. Increasing metabolic rate is energetically expensive. This may be a physiological response developed to cope with stressful conditions in the short-term but could also be an involuntary change caused by a speed-up of biochemical reactions. Irrespective of the mechanism, this suggests a higher energy demand necessary for the maintenance, active metabolism, and overall survival of oysters. However, long-term elevation in SMR may not be sustainable for organisms due to the added energetic costs, particularly if left uncompensated, unless they become adapted over multiple generations.

4.2. Clearance rate

In the literature, the terms feeding rate, ingestion rate, clearance rate, and filtering rate are often used in concomitance or interchangeably (e.g. Coughlan, 1969; Fernández-Reiriz et al., 2011; Sanders et al., 2013). All are related to the amount of particles or the volume of water being processed over time. Previous studies have shown that respiration and feeding in oysters are related (Giomi et al., 2016; Haure et al., 1995, 2003). Higher metabolism leads to higher energetic demands, commonly met through enhanced food consumption. Here however, contrary to predictions, no relationships between respiration and feeding rates were found for either species. Nevertheless, in our study, the clearance rate of *M. gigas* followed an increasing trend under elevated temperature, particularly at ambient and intermediate pCO_2 (Fig. 4.), suggesting a mechanism working towards enhanced food acquisition and energy supply. While increased feeding activity with temperature has been previously shown in several species of mollusc (e.g: *O. edulis* (non-linear increase from 10 °C to 30 °C - Haure et al., 1998, and references therein), *M. galloprovinciallis* (from 12 °C to 18 °C - Kroeker et al., 2014), and *Mytilus chilensis* (between 12 °C and 16 °C - Navarro et al., 2016)), it was observed in *O. edulis* in this study only after 10 days of exposure, following which clearance rates returned to control levels. This suggests an initial acclimation response to experimental conditions rather than a longer-term response to the treatment.

Elevated pCO_2 reduced the clearance rate of *M. gigas* by up to 40%; an effect not observed in *O. edulis*. There is a burgeoning literature on the effects of elevated pCO_2 on the feeding behaviour and clearance rate of juvenile and adult bivalves, both of which are increasingly recognised as potential key physiological traits that govern an organisms' responses to ocean acidification (Vargas et al., 2015). Although feeding is an energetically expensive process (Pörtner et al., 2004), it has the potential to alleviate the negative effects of ocean acidification by providing the required additional energy to overcome the increased cost of metabolism. Indeed, several studies have shown that high food availability can counteract the effects of acidification on molluscan larvae and juveniles (Hettinger et al., 2013a; Sanders et al., 2013; Thomsen et al., 2012). However, elevated pCO_2 has also been shown to negatively impact on the clearance and ingestion rates of several species of molluscs as found here for *M. gigas*. Juveniles of the mussel *Perumytilus purpuratus* decreased their clearance rates by up to 70% under similar pCO_2 levels (at 700 ppm and 1000 ppm - Vargas et al., 2015). Elevated pCO_2 to 1000 ppm also led to reduced clearance rate and absorption efficiency in *M. chilensis* (Navarro et al., 2016), and to a weak decrease in feeding rates in *M. galloprovinciallis* at 1200 ppm (Kroeker et al., 2014). Additionally, more extreme pCO_2 levels have also been linked to reduced clearance and ingestion rates in juveniles of the clam *R. decussatus* (between 1698 ppm and 4345 ppm - Fernández-Reiriz et al., 2011). Impairment of filtration and feeding can prevent organisms from resisting ocean acidification or compensating for its effects, with subsequent starvation leading to increased mortality within the population. In accordance with our results for *O. edulis,* no marked effects of elevated pCO_2 on clearance rate were recorded in *P.*

maximus (at levels up to 1140 ppm - Sanders et al., 2013). These results reinforce the idea that responses to acidification conditions are not only species-specific, but also dependent on the range and number of pCO_2 levels considered.

4.3. Condition index

The higher metabolic costs associated with increased respiration rates under future OAW conditions, particularly in *M. gigas*, were not compensated for by added energy through enhanced feeding. However, added energetic demands can also be met by other trade-offs with calcification, reproduction, and growth of somatic tissues.

Condition Indices (CI) are recognised as useful tools to evaluate the overall status and health of bivalves (Knights, 2012), and reflect their ability to withstand adverse environmental conditions (Marin et al., 2003). Stressful environmental conditions requiring significant energetic expenditure result in low CI in bivalves over time (Orban et al., 2002). Here, the CI of *M. gigas* was negatively impacted by elevated temperature but not elevated pCO_2, an effect also seen for the mussel *M. edulis* (Mackenzie et al., 2014). Our results for pCO_2-exposed individuals are in contrast to those of Lannig et al. (2010) on *M. gigas* who recorded a decrease of ~20% in CI between control individuals and those exposed to elevated pCO_2. However, similar decreases in CI with elevated temperature were recorded in several other bivalves (Gabbott and Walker, 1971; Hiebenthal et al., 2012; Shpigel et al., 1992). Bivalves have the capacity to reallocate energy reserves by reabsorbing somatic tissues and gonads to sustain routine maintenance when needed. Declines in CI usually suggest depletion of these reserves and are often associated with long-term stressful conditions (Lannig et al., 2010) or alterations in energy budget (Melzner et al., 2009).

As reduced condition index is associated with depletion of energetic reserves, it suggests that the long-term costs associated with increased metabolism in *M. gigas* were met by a reallocation of reserves from somatic and gonadal tissues to sustain maintenance and insure survival. While no mortality of *M. gigas* occurred during the experiment, the lack of acclimation in respiration and clearance rates responses after 12 weeks exposure suggests that, if left uncompensated, the added metabolic costs could compromise survival once all somatic and gonadal reserves are depleted.

The CI of *O. edulis* was unaffected by any of the treatment conditions, suggesting that the experimental environmental conditions were not equally experienced by both species, and *M. gigas* only may be stressed. A potential explanation for the maintenance of *O. edulis* CI when exposed to the elevated temperature and 1000 ppm pCO_2 treatment despite increased metabolic rates is that its sustained clearance rates provided sufficient energy supply to compensate for the additional metabolic costs over the 12 weeks. Nevertheless, exposure beyond the 12 week period of this study might produce *O. edulis* displaying lowered CI from longer-term accumulated and uncompensated energetic costs.

5. Conclusion

This study has shown that two important physiological traits of oysters are affected by warming and/or acidification, however the responses appear species-specific. Due to logistic limitations inherent to the OAW system used during the experiment, the sample size for each species was limited to n = 4 per treatment and as such, there was high variability in the responses recorded, which led to lack of statistical power for the analysis. Yet despite this, clear biological effects were apparent. If anthropogenic CO_2 emissions continue to rise and temperatures continue to increase, increased metabolic cost to oysters are predicted. *Magallana gigas* in particular may find it difficult to meet these costs due to decreased feeding activity at 750 ppm pCO_2 levels. Non-native and invasive species are often more resilient to environmental fluctuations and other biotic or abiotic stressors, yet in oysters sampled from wild Plymouth populations, *M. gigas* was more negatively impacted by the OAW scenarios tested than its native counterpart, *O. edulis* – which contradicted our initial predictions. The non-native oysters had elevated metabolism, reduced feeding, and decreased condition, signs that it could not cope well with the warming and acidification conditions. Krassoi et al. (2008) demonstrated that differences exist with respect to abiotic environmental tolerances of extreme physical conditions between exotic and native oyster species, with the native species able to withstand harsher environmental conditions. This was also recently observed in Brazil, where the native *Crassostrea brasiliana* was more tolerant to high temperatures than the non-native *M. gigas* (Moreira et al., 2017). However, it should be noted that, although here only two factors were tested, the interaction of multiple environmental drivers has been shown to influence the sensitivity of organisms to a single specific factor (Parker et al., 2017a; Parker et al., 2017b).

Due to poorer performance and condition of individual *M. gigas*, as found here, warming and acidification may threaten populations maintenance and functioning, degrading the provision of ecosystem services such as erosion control, improved water quality, and fisheries from unharvested wild beds, while reducing aquaculture productivity at designated aquaculture sites. The latter is especially important in the UK where harvest of cultured *M. gigas* populations constitutes 90% of the oyster aquaculture production, worth an estimated £10.14 million annually (Humphreys et al., 2014). Additionally, reduced clearance rates of *M. gigas* under OAW may have important ecological impacts by limiting their ability to reduce turbidity and improve water quality. Similar concerns have been expressed regarding the fate of waste bioremediation service by mussels under future ocean acidification, as their filtration rates might be negatively impacted (Broszeit et al., 2015). Wild unharvested oyster beds consisting in majority of *M. gigas* might see their surrounding water quality diminish, with negative consequences for further associated ecosystem services such as allowing for recreational use and promoting the maintenance of submerged vegetation. In contrast, it appears that under future OAW corresponding to the levels tested in this study, *O. edulis* will be able to continue delivering its important bio-filtration service, and consequently the provision of improved water quality will remain secure, if abundances recover and beds become functional again.

Such findings are of importance in terms of species ecological status, population conservation, and management measures. Oyster-related ecosystem services are mostly associated with 'reef' formations, which would require high recruitment and abundant populations (Herbert et al., 2012). As such, further efforts to promote the restoration of native *O. edulis* beds should be pursued, and efforts to eradicate *M. gigas* populations may be reconsidered, in order to secure not only food provision, but also good water quality and associated beneficial ecosystem services in the future from functional populations of both species. However, ecological and economic trade-offs will need to be considered carefully, as the delivery of some of these ecosystem services from wild populations (food provision *vs* water quality) may be at odds given their opposing effects on oyster abundances.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.marenvres.2018.10.003.

References

Airoldi, L., Beck, M.W., 2007. Loss, status and trends for coastal marine habitats of Europe. Oceanogr. Mar. Biol. Annu. Rev. 45, 345–405.

Angles d'Auriac, M.B., Rinde, E., Norling, P., Lapegue, S., Staalstrom, A., Hjermann, D.O., et al., 2017. Rapid expansion of the invasive oyster *Crassostrea gigas* at its northern distribution limit in Europe: naturally dispersed or introduced? PloS One 12 (5), e0177481. https://doi.org/10.1371/journal.pone.0177481.

Artigaud, S., Lacroix, C., Pichereau, V., Flye-Sainte-Marie, J., 2014. Respiratory response to combined heat and hypoxia in the marine bivalves *Pecten maximus* and *Mytilus* spp. Comp. Biochem. Physiol. Mol. Integr. Physiol. 175, 135–140. https://doi.org/10.1016/j.cbpa.2014.06.005.

Barry, J.P., Widdicombe, S., Hall-Spencer, J.M., 2011. Chapter 10: effects of ocean acidification on marine biodiversity and ecosystem function. Ocean Acidification 192–209.

Barton, A., Waldbusser, G., Feely, R., Weisberg, S., Newton, J., Hales, B., et al., 2015. Impacts of coastal acidification on the Pacific Northwest shellfish industry and adaptation strategies implemented in response. Oceanography 25 (2), 146–159. https://doi.org/10.5670/oceanog.2015.38.

Beck, M.W., Brumbaugh, R.D., Airoldi, L., Carranza, A., Coen, L.D., Crawford, C., et al., 2011. Oyster reefs at risk and recommendations for conservation, restoration, and management. Bioscience 61 (2), 107–116. https://doi.org/10.1525/bio.2011.61.2.5.

Beiras, R., Camacho, A.P., Albentosa, M., 1995. Short-term and long-term alterations in the energy budget of young oyster *Ostrea edulis* L. in response to temperature change. J. Exp. Mar. Biol. Ecol. 186 (2), 221–236. https://doi.org/10.1016/0022-0981(94)00159-B.

Beniash, E., Ivanina, A., Lieb, N.S., Kurochkin, I., Sokolova, I.M., 2010. Elevated level of carbon dioxide affects metabolism and shell formation in oysters *Crassostrea virginica* (Gmelin). Mar. Ecol. Prog. Ser. 419, 95–108. https://doi.org/10.3354/meps08841.

Bertness, M.D., Gaines, S.D., 1993. Larval dispersal and local adaptation in acorn barnacles. Evolution 47 (1), 316–320.

Bodoy, A., Prou, J., Berthome, J.-P., 1986. Etude comparative de différents indices de condition chez l'huître creuse (*Crassostrea gigas*). Haliotis 15, 173–182.

Bougrier, S., Collet, B., Geairon, P., Geffard, O., He´ral, M., 1998. Respiratory time activity of the Japanese oyster *Crassostrea gigas* (Thunberg). J. Exp. Mar. Biol. Ecol. 219, 205–216.

Broszeit, S., Hattam, C., Beaumont, N., 2015. Bioremediation of waste under ocean acidification: reviewing the role *of Mytilus edulis*. Mar. Pollut. Bull. https://doi.org/10.1016/j.marpolbul.2015.12.040.

Buxton, C., Newell, R., Field, J., 1981. Response-surface analysis of the combined effects of exposure and acclimation temperatures on filtration, oxygen consumption and scope for growth in the oyster *Ostrea edulis*. Mar. Ecol. Prog. Ser. 6 (1), 73–82.

Byrne, M., Przeslawski, R., 2013. Multistressor impacts of warming and acidification of the ocean on marine invertebrates' life histories. Integr. Comp. Biol. 53 (4), 582–596. https://doi.org/10.1093/icb/ict049.

Calosi, P., Turner, L.M., Hawkins, M., Bertolini, C., Nightingale, G., Truebano, M., et al., 2013. Multiple physiological responses to multiple environmental challenges: an individual approach. Integr. Comp. Biol. 53 (4), 660–670. https://doi.org/10.1093/icb/ict041.

Coen, L.D., Brumbaugh, R.D., Bushek, D., Grizzle, R., Luckenbach, M.W., Posey, M.H., et al., 2007. Ecosystem services related to oyster restoration. Mar. Ecol. Prog. Ser. 341, 303–307.

Cole, V.J., Parker, L.M., O'Connor, S.J., O'Connor, W.A., Scanes, E., Byrne, M., et al., 2016. Effects of multiple climate change stressors: ocean acidification interacts with warming, hyposalinity, and low food supply on the larvae of the brooding flat oyster *Ostrea angasi*. Mar. Biol. 163 (5). https://doi.org/10.1007/s00227-016-2880-4.

Comeau, L.A., Pernet, F., Tremblay, R., Bates, S.S., LeBlanc, A., 2008. Comparison of Eastern Oyster (*Crassostrea Virginica*) and Blue Mussel (*Mytilus edulis*) Filtration Rates at Low Temperatures: Fisheries and Oceans Canada.

Coolen, J.W.P., 2017. North Sea Reefs. Benthic Biodiversity of Artificial and Rocky Reefs in the Southern North Sea. Wageningen University.

Cooley, S.R., Cheney, J.E., Kelly, R.P., Allison, E.H., 2017. 2 Ocean acidification and Pacific oyster larval failures in the Pacific Northwest United States. In: Global Change in Marine Systems: Societal and Governing Responses.

R Core Team, 2016. R: a Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria Available from: https://www.R-project.org/.

Coughlan, J., 1969. The estimation of filtering rate from the clearance of suspensions. Mar. Biol. 2 (4), 356–358. https://doi.org/10.1007/bf00355716.

Davis, C.H., Calabrese, A., 1969. Survival and growth of larvae of the European oyster (*Ostrea edulis* L.) at different temperatures. Biol. Bull. 136 (2), 193–199. https://doi.org/10.2307/1539813.

Dickinson, G.H., Ivanina, A.V., Matoo, O.B., Portner, H.O., Lannig, G., Bock, C., et al., 2012. Interactive effects of salinity and elevated CO_2 levels on juvenile eastern oysters, *Crassostrea virginica*. J. Exp. Biol. 215 (Pt 1), 29–43. https://doi.org/10.1242/jeb.061481.

Dickson, A.G., 1990. Thermodynamics of the dissociation of boric acid in synthetic seawater from 273.15 to 318.15 K. Deep-Sea Res. Part a-Oceanographic Res. Pap. 37 (755), 66.

Dickson, A.G., Millero, F.J., 1987. A comparison of the equilibrium constants for the dissociation of carbonic acid in seawater media. Deep-Sea Res. Part a-Oceanographic Res. Pap. 34 (1733), 43.

Dolmer, P., Holm, M.W., Strand, Å., Lindegarth, S., Bodvin, T., Norling, P., et al., 2014. The invasive Pacific oyster, *Crassostrea gigas*. In: Scandinavian Coastal Waters: a Risk Assessment on the Impact in Different Habitats and Climate Conditions. Institute of Marine Research, pp. 0071–5638.

Dove, M.C., Sammut, J., 2007. Impacts of estuarine acidification on survival and growth of Sydney rock oysters *Saccostrea glomerata* (Gould 1850). J. Shellfish Res. 26 (2), 519–527.

Fernández-Reiriz, M.J., Range, P., Álvarez-Salgado, X.A., Labarta, U., 2011. Physiological energetics of juvenile clams *Ruditapes decussatus* in a high CO_2 coastal ocean. Mar. Ecol. Prog. Ser. 433, 97–105. https://doi.org/10.3354/meps09062.

Gabbott, P.A., Walker, A.J.M., 1971. Changes in the condition index and biochemical content of adult oysters (*Ostrea edulis* L.) maintained under hatchery conditions. J. Conseil 34 (1), 98–105. https://doi.org/10.1093/icesjms/34.1.98.

Gazeau, F., Alliouane, S., Correa, M.L., Gentile, M., Bock, C., Hirse, T., et al., 2014. Impact of ocean acidification and warming on the Mediterranean mussel (*Mytilus galloprovincialis*). Front. Mar. Sci. 1, 62. https://doi.org/10.3389/fmars.2014.00062.

Gestoso, I., Arenas, F., Olabarria, C., 2016. Ecological interactions modulate responses of two intertidal mussel species to changes in temperature and pH. J. Exp. Mar. Biol. Ecol. 474, 116–125. https://doi.org/10.1016/j.jembe.2015.10.006.

Giomi, F., Mandaglio, C., Ganmanee, M., Han, G.D., Dong, Y.W., Williams, G.A., et al., 2016. The importance of thermal history: costs and benefits of heat exposure in a tropical, rocky shore oyster. J. Exp. Biol. 219 (Pt 5), 686–694. https://doi.org/10.1242/jeb.128892.

Gray, M.W., Langdon, C.J., Waldbusser, G.G., Hales, B., Kramer, S., 2017. Mechanistic understanding of ocean acidification impacts on larval feeding physiology and energy budgets of the mussel *Mytilus californianus*. Mar. Ecol. Prog. Ser. 563, 81–94. https://doi.org/10.3354/meps11977.

Green, E.J., Carritt, D.E., 1967. New tables for oxygen saturation of seawater. J. Mar. Res. 25, 140–147.

Hales, B., Suhrbier, A., Waldbusser, G.G., Feely, R.A., Newton, J.A., 2016. The carbonate chemistry of the “fattening line,” Willapa Bay, 2011–2014. Estuar. Coast 40 (1), 173–186. https://doi.org/10.1007/s12237-016-0136-7.

Hall-Spencer, J., Allen, R., 2015. The impact of CO_2 emissions on 'nuisance' marine species. Res. Rep. Biodivers. Stud. 33, 33–46. https://doi.org/10.2147/rrbs.s70357.

Harding, S., Nelson, L., Glover, T., 2016. Solent Oyster Restoration Project Management Plan. Blue Marine Foundation.

Harvey, B.P., Gwynn-Jones, D., Moore, P.J., 2013. Meta-analysis reveals complex marine biological responses to the interactive effects of ocean acidification and warming. Ecol. Evol. 3https://doi.org/10.1002/ece3.728. 2782-2782.

Haure, J., Penisson, C., Bougrier, S., Baud, J.P., 1995. Allometric relationships and effects of temperature on clearance and oxygen consumption rates of *Crassostrea gigas*. Aquaculture 169, 211–224.

Haure, J., Penisson, C., Bougrier, S., Baud, J., 1998. Influence of temperature on clearance and oxygen consumption rates of the flat oyster *Ostrea edulis*: determination of allometric coefficients. Aquaculture 169 (3), 211–224.

Haure, J., Huvet, A., Palvadeau, H., Nourry, M., Penisson, C., Martin, J.L.Y., et al., 2003. Feeding and respiratory time activities in the cupped oysters *Crassostrea gigas, Crassostrea angulata* and their hybrids. Aquaculture 218 (1–4), 539–551. https://doi.org/10.1016/s0044-8486(02)00493-3.

Herbert, R.J.H., Robert, C., Humphreys, J., Fletcher, S., 2012. The Pacific Oyster (Crassostrea gigas) in the UK: Economic, Legal and Environmental Issues Associated with its Cultivation, Wild Establishment and Exploitation. Report for the Shellfish Association of Great Britain.

Herbert, R.J.H., Humphreys, J., Davies, C.J., Roberts, C., Fletcher, S., Crowe, T.P., 2016. Ecological impacts of non-native Pacific oysters (*Crassostrea gigas*) and management measures for protected areas in Europe. Biodivers. Conserv. 25 (14), 2835–2865. https://doi.org/10.1007/s10531-016-1209-4.

Hettinger, A., Sanford, E., Hill, T.M., Russell, A.D., Sato, K.N.S., Hoey, J., et al., 2012. Persistent carry-over effects of planktonic exposure to ocean acidification in the Olympia oyster. Ecology 93 (12), 2758–2768. https://doi.org/10.1890/12-0567.1.

Hettinger, A., Sanford, E., Hill, T., Hosfelt, J., Russell, A., Gaylord, B., 2013a. The influence of food supply on the response of Olympia oyster larvae to ocean acidification. Biogeosciences 10 (10), 6629–6638.

Hettinger, A., Sanford, E., Hill, T.M., Lenz, E.A., Russell, A.D., Gaylord, B., 2013b. Larval carry-over effects from ocean acidification persist in the natural environment. Global Change Biol. 19 (11), 3317–3326. https://doi.org/10.1111/gcb.12307.

Hiebenthal, C., Philipp, E., Eisenhauer, A., Wahl, M., 2012. Interactive effects of temperature and salinity on shell formation and general condition in Baltic Sea *Mytilus edulis* and *Arctica islandica*. Aquat. Biol. 14, 289–298.

Hofmann, G.E., Barry, J.P., Edmunds, P.J., Gates, R.D., Hutchins, D.A., Klinger, T., et al., 2010. The effect of ocean acidification on calcifying organisms in marine ecosystems: an organism-to-ecosystem perspective. Annu. Rev. Ecol. Evol. Syst. 41 (1), 127–147. https://doi.org/10.1146/annurev.ecolsys.110308.120227.

Houlbrèque, F., Reynaud, S., Godinot, C., Oberhänsli, F., Rodolfo-Metalpa, R., Ferrier-Pagès, C., 2015. Ocean acidification reduces feeding rates in the scleractinian coral *Stylophora pistillata*. Limnol. Oceanogr. 60 (1), 89–99. https://doi.org/10.1002/lno.10003.

Humphreys, M.P., 2016. Climate sensitivity and the rate of ocean acidification: future impacts, and implications for experimental design. ICES J. Mar. Sci. 74 (4), 934–940.

https://doi.org/10.1093/icesjms/fsw189.
Humphreys, J., Herbert, R.J.H., Roberts, C., Fletcher, S., 2014. A reappraisal of the history and economics of the Pacific oyster in Britain. Aquaculture 428–429, 117–124. https://doi.org/10.1016/j.aquaculture.2014.02.034.
Knights, A.M., 2012. Spatial variation in body size and reproductive condition of subtidal mussels: considerations for sustainable management. Fish. Res. 113 (1), 45–54. https://doi.org/10.1016/j.fishres.2011.09.002.
Kochmann, J., O'Beirn, F., Yearsley, J., Crowe, T.P., 2013. Environmental factors associated with invasion: modelling occurrence data from a coordinated sampling programme for Pacific oysters. Biol. Invasions 15 (10), 2265–2279.
Krassoi, F.R., Brown, K.R., Bishop, M.J., Kelaher, B.P., Summerhayes, S., 2008. Condition-specific competition allows coexistence of competitively superior exotic oysters with native oysters. J. Anim. Ecol. 77 (1), 5–15. https://doi.org/10.1111/j.1365-2656.2007.01316.x.
Kroeker, K.J., Kordas, R.L., Crim, R., Hendriks, I.E., Ramajo, L., Singh, G.S., et al., 2013. Impacts of ocean acidification on marine organisms: quantifying sensitivities and interaction with warming. Global Change Biol. 19 (6), 1884–1896. https://doi.org/10.1111/gcb.12179.
Kroeker, K.J., Gaylord, B., Hill, T.M., Hosfelt, J.D., Miller, S.H., Sanford, E., 2014. The role of temperature in determining species' vulnerability to ocean acidification: a case study using *Mytilus galloprovincialis*. PloS One 9 (7), e100353. https://doi.org/10.1371/journal.pone.0100353.
Kurihara, H., 2008. Effects of CO_2-driven ocean acidification on the early developmental stages of invertebrates. Mar. Ecol. Prog. Ser. 373, 275–284. https://doi.org/10.3354/meps07802.
Lacoue-Labarthe, T., Nunes, P.A.L.D., Ziveri, P., Cinar, M., Gazeau, F., Hall-Spencer, J.M., et al., 2016. Impacts of ocean acidification in a warming Mediterranean Sea: an overview. Reg. Stud. Marine Sci. 5, 1–11. https://doi.org/10.1016/j.rsma.2015.12.005.
Laing, I., Walker, P., Areal, F., 2006. Return of the native – is European oyster (*Ostrea edulis*) stock restoration in the UK feasible? Aquat. Living Resour. 19 (3), 283–287. https://doi.org/10.1051/alr:2006029.
Lallias, D., Boudry, P., Lapègue, S., King, J., Beaumont, A., 2010. Strategies for the retention of high genetic variability in European flat oyster (*Ostrea edulis*) restoration programmes. Conserv. Genet. 11 (5), 1899–1910. https://doi.org/10.1007/s10592-010-0081-0.
Lannig, G., Eilers, S., Portner, H.O., Sokolova, I.M., Bock, C., 2010. Impact of ocean acidification on energy metabolism of oyster, *Crassostrea gigas*-changes in metabolic pathways and thermal response. Mar. Drugs 8 (8), 2318–2339. https://doi.org/10.3390/md8082318.
Lemasson, A.J., Fletcher, S., Hall-Spencer, J.M., Knights, A.M., 2017. Linking the biological impacts of ocean acidification on oysters to changes in ecosystem services: a review. J. Exp. Mar. Biol. Ecol. 492, 49–62. https://doi.org/10.1016/j.jembe.2017.01.019.
Leung, J.Y., Russell, B.D., Connell, S.D., 2017. Mineralogical plasticity acts as a compensatory mechanism to the impacts of ocean acidification. Environ. Sci. Technol. https://doi.org/10.1021/acs.est.6b04709.
Liu, S., Shi, W., Guo, C., Zhao, X., Han, Y., Peng, C., et al., 2016. Ocean acidification weakens the immune response of blood clam through hampering the NF-kappa beta and toll-like receptor pathways. Fish Shellfish Immunol. 54, 322–327. https://doi.org/10.1016/j.fsi.2016.04.030.
Lodge, D.M., 1993. Biological invasions: lessons for ecology. Trends Ecol. Evol. 8 (4), 133–137.
Lucas, A., Beninger, P.G., 1985. The use of physiological condition indices in marine bivalve aquaculture. Aquaculture 44 (3), 187–200. https://doi.org/10.1016/0044-8486(85)90243-1.
Mackenzie, C.L., Ormondroyd, G.A., Curling, S.F., Ball, R.J., Whiteley, N.M., Malham, S.K., 2014. Ocean warming, more than acidification, reduces shell strength in a commercial shellfish species during food limitation. PloS One 9 (1), e86764. https://doi.org/10.1371/journal.pone.0086764.
Marin, M.G., Moschino, V., Deppieri, M., Lucchetta, L., 2003. Variations in gross biochemical composition, energy value and condition index of *T. philippinarum* from the Lagoon of Venice. Aquaculture 219 (1–4), 859–871. https://doi.org/10.1016/s0044-8486(03)00035-8.
Matoo, O.B., Ivanina, A.V., Ullstad, C., Beniash, E., Sokolova, I.M., 2013. Interactive effects of elevated temperature and CO_2 levels on metabolism and oxidative stress in two common marine bivalves (*Crassostrea virginica* and Mercenaria mercenaria). Comp. Biochem. Physiol. Mol. Integr. Physiol. 164 (4), 545–553. https://doi.org/10.1016/j.cbpa.2012.12.025.
Mehrbach, C., Culberson, C.H., Hawley, J.E., R.M.P, 1973. Measurement of the apparent dissociation constants of carbonic acid in seawater at atmospheric pressure. Limnol. Oceanogr. 18 (897), 907.
Melzner, F., Gutowska, M., Langenbuch, M., Dupont, S., Lucassen, M., Thorndyke, M.C., et al., 2009. Physiological basis for high CO_2 tolerance in marine ectothermic animals: pre-adaptation through lifestyle and ontogeny? Biogeosciences 6 (10), 2313–2331.
Moreira, A., Figueira, E., Pecora, I.L., Soares, A.M., Freitas, R., 2017. Biochemical alterations in native and exotic oyster species in Brazil in response to increasing temperature. Comp. Biochem. Physiol. C Toxicol. Pharmacol. 191, 183–193. https://doi.org/10.1016/j.cbpc.2016.10.008. PubMed PMID: 27816652.
Moreira, A., Figueira, E., Pecora, I.L., Soares, A., Freitas, R., 2018. Native and exotic oysters in Brazil: comparative tolerance to hypercapnia. Environ. Res. 161, 202–211. https://doi.org/10.1016/j.envres.2017.10.035.
Navarro, J.M., Duarte, C., Manríquez, P.H., Lardies, M.A., Torres, R., Acuña, K., et al., 2016. Ocean warming and elevated carbon dioxide: multiple stressor impacts on juvenile mussels from southern Chile. ICES J. Mar. Sci. 73 (3), 764–771. https://doi.org/10.1093/icesjms/fsv249.
Orban, E., Di Lena, G., Nevigato, T., Casini, I., Marzetti, A., Caproni, R., 2002. Seasonal changes in meat content, condition index and chemical composition of mussels (*Mytilus galloprovincialis*) cultured in two different Italian sites. Food Chem. 77, 57–65.
Orton, J.H., 1937. Oyster Biology and Oyster Culture. Butler and Tanner Ltd, London. UK.
Orton, J.H., 1940. Effect of the severe frost of the winter of 1939-40 on the Fauna of the Essex oyster beds. Nature 145, 708–709.
Osborn, D., Dupont, S., Hansson, L., Metian, M., 2017. Chapter 19. Ocean Acidification: Impacts and Governance. Handbook on the Economics and Management of Sustainable Oceans. Edward Elgar Publishing, pp. 396.
Parker, L.M., Ross, P.M., O'Connor, W.A., 2010. Comparing the effect of elevated pCO_2 and temperature on the fertilization and early development of two species of oysters. Mar. Biol. 157 (11), 2435–2452. https://doi.org/10.1007/s00227-010-1508-3.
Parker, L.M., Ross, P.M., O'Connor, W.A., Portner, H.O., Scanes, E., Wright, J.M., 2013. Predicting the response of molluscs to the impact of ocean acidification. Biology 2 (2), 651–692. https://doi.org/10.3390/biology2020651.
Parker, L.M., O'Connor, W.A., Byrne, M., Coleman, R.A., Virtue, P., Dove, M., et al., 2017a. Adult exposure to ocean acidification is maladaptive for larvae of the Sydney rock oyster *Saccostrea glomerata* in the presence of multiple stressors. Biol. Lett. 13 (2). https://doi.org/10.1098/rsbl.2016.0798.
Parker, L.M., Scanes, E., O'Connor, W.A., Coleman, R.A., Byrne, M., Portner, H.O., et al., 2017b. Ocean acidification narrows the acute thermal and salinity tolerance of the Sydney rock oyster *Saccostrea glomerata*. Mar. Pollut. Bull. 122 (1–2), 263–271. https://doi.org/10.1016/j.marpolbul.2017.06.052.
Pierrot, D., Lewis, E., DWR, W., 2006. MS Excel Program Developed for CO2 System Calculations, ORNL/CDIAC-105. Oak Ridge National Laboratory, U.S. Department of Energy. Carbon Dioxide Information Analysis Center, Oak Ridge (TN).
Pörtner, H.O., Farrell, A.P., 2008. Physiology and climate change. Science 322 (5902), 690–692. https://doi.org/10.1126/science.1163156.
Pörtner, H., Langenbuch, M., Reipschläger, A., 2004. Biological impact of elevated ocean CO_2 concentrations: lessons from animal physiology and Earth history. J. Oceanogr. 60 (4), 705–718. https://doi.org/10.1007/s10872-004-5763-0.
Pörtner, H.-O., Karl, D.M., Boyd, P.W., Cheung, W.W.L., Lluch-Cota, S.E., Nojiri, Y., et al., 2014. Ocean Systems. Climate Change 2014: Impacts, Adaptation, and Vulnerability Part A: Global and Sectoral Aspects Contribution of Working Group II to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change. Cambridge University Press, Cambridge, United Kingdom and New York, NY, USA, pp. 411–484.
Przeslawski, R., Byrne, M., Mellin, C., 2015. A review and meta-analysis of the effects of multiple abiotic stressors on marine embryos and larvae. Global Change Biol. 21 (6), 2122–2140. https://doi.org/10.1111/gcb.12833.
Ramajo, L., Marba, N., Prado, L., Peron, S., Lardies, M.A., Rodriguez-Navarro, A., et al., 2015. Biomineralization changes with food supply confer juvenile scallops (*Argopecten purpuratus*) resistance to ocean acidification. Global Change Biol. https://doi.org/10.1111/gcb.13179.
Reum, J.C.P., Alin, S.R., Harvey, C.J., Bednaršek, N., Evans, W., Feely, R.A., et al., 2015. Interpretation and design of ocean acidification experiments in upwelling systems in the context of carbonate chemistry co-variation with temperature and oxygen. ICES J. Mar. Sci. 73 (3), 582–595. https://doi.org/10.1093/icesjms/fsu231.
Rinde, E., Tjomsland, T., Hjermann, D., Kempa, M., Norling, P., Kolluru, V.S., 2016. Increased spreading potential of the invasive Pacific oyster (*Crassostrea gigas*) at its northern distribution limit in Europe due to warmer climate. Mar. Freshw. Res. https://doi.org/10.1071/mf15071.
Robert, R., Vignier, J., Petton, B., 2017. Influence of feeding regime and temperature on development and settlement of oyster *Ostrea edulis* (Linnaeus, 1758) larvae. Aquacult. Res. 48 (9), 4756–4773. https://doi.org/10.1111/are.13297.
Sanders, M.B., Bean, T.P., Hutchinson, T.H., Le Quesne, W.J., 2013. Juvenile king scallop, Pecten maximus, is potentially tolerant to low levels of ocean acidification when food is unrestricted. PloS One 8 (9), e74118. https://doi.org/10.1371/journal.pone.0074118.
Sanford, E., Kelly, M.W., 2011. Local adaptation in marine invertebrates. Ann. Rev. Mar. Sci. 3, 509–535. https://doi.org/10.1146/annurev-marine-120709-142756.
Saucedo, P.E., Ocampo, La, Monteforte, M., Bervera, H., 2004. Effect of temperature on oxygen consumption and ammonia excretion in the Calafia mother-of-pearl oyster, *Pinctada mazatlanica* (Hanley, 1856). Aquaculture 229 (1–4), 377–387. https://doi.org/10.1016/s0044-8486(03)00327-2.
Sawusdee, A., 2015. PhD Thesis. Restoration of the European Flat Oyster *Ostrea edulis* Using Elevated Broodstock Reefs. University of Southampton.
Scanes, E., Parker, L.M., O'Connor, W.A., Stapp, L.S., Ross, P.M., 2017. Intertidal oysters reach their physiological limit in a future high-CO_2 world. J. Exp. Biol. 220 (Pt 5), 765–774. https://doi.org/10.1242/jeb.151365.
Shelmerdine, R.L., Leslie, B., 2009. Restocking of the native oyster, *Ostrea edulis*. In: Shetland: Habitat Identification Study Scottish Natural Heritage Commissioned Report No. 396.
Shelmerdine, R., Mouat, B., Shucksmith, R., 2017. The most northerly record of feral Pacific oyster *Crassostrea gigas* (Thunberg, 1793) in the British Isles. BioInvasions Records 6 (1), 57–60. https://doi.org/10.3391/bir.2017.6.1.09.
Shpigel, M., Barber, B.J., Mann, R., 1992. Effects of elevated temperature on growth, gametogenesis, physiology, and biochemical composition in diploid and triploid Pacific oysters, *Crassostrea gigas* Thunberg. J. Exp. Mar. Biol. Ecol. 161 (1), 15–25. https://doi.org/10.1016/0022-0981(92)90186-E.
Stachowicz, J.J., Fried, H., Osman, R.W., Whitlatch, R.B., 2002. Biodiversity, invasion resistance, and marine ecosystem function: reconciling pattern and process. Ecology 83 (9), 2575–2590.
Sunday, J.M., Fabricius, K.E., Kroeker, K.J., Anderson, K.M., Brown, N.E., Barry, J.P., et al., 2016. Ocean acidification can mediate biodiversity shifts by changing biogenic

habitat. Nat. Clim. Change 7 (1), 81–85. https://doi.org/10.1038/nclimate3161.
Thomas, Y., Pouvreau, S., Alunno-Bruscia, M., Barillé, L., Gohin, F., Bryère, P., et al., 2016. Global change and climate-driven invasion of the Pacific oyster (*Crassostrea gigas*) along European coasts: a bioenergetics modelling approach. J. Biogeogr. 43 (3), 568–579. https://doi.org/10.1111/jbi.12665.
Thomsen, J., Melzner, F., 2010. Moderate seawater acidification does not elicit long-term metabolic depression in the blue mussel *Mytilus edulis*. Mar. Biol. 157 (12), 2667–2676.
Thomsen, J., Casties, I., Pansch, C., Kortzinger, A., Melzner, F., 2012. Food availability outweighs ocean acidification effects in juvenile *Mytilus edulis*: laboratory and field experiments. Global Change Biol. 19 (4), 1017–1027. https://doi.org/10.1111/gcb.12109.
Towle, E.K., Enochs, I.C., Langdon, C., 2015. Threatened Caribbean coral is able to mitigate the adverse effects of ocean acidification on calcification by increasing feeding rate. PloS One 10 (4), e0123394. https://doi.org/10.1371/journal.pone.0123394.
Townhill, B., Pinnegar, J., Tinker, J., Jones, M., Simpson, S., Stebbing, P., et al., 2017. Non-native marine species in north-west Europe: developing an approach to assess future spread using regional downscaled climate projections. Aquat. Conserv. Mar. Freshw. Ecosyst. 27 (5), 1035–1050. https://doi.org/10.1002/aqc.2764.
Troost, K., 2010. Causes and effects of a highly successful marine invasion: case-study of the introduced Pacific oyster *Crassostrea gigas* in continental NW European estuaries. J. Sea Res. 64 (3), 145–165. https://doi.org/10.1016/j.seares.2010.02.004.
Vargas, C.A., Aguilera, V.M., Martín, V.S., Manríquez, P.H., Navarro, J.M., Duarte, C., et al., 2015. CO_2-driven ocean acidification disrupts the filter feeding behavior in Chilean gastropod and bivalve species from different geographic localities. Estuar. Coast 38 (4), 1163–1177. https://doi.org/10.1007/s12237-014-9873-7.
Waldbusser, G.G., Steenson, R.A., Green, M.A., 2011a. Oyster shell dissolution rates in estuarine waters: effects of pH and shell legacy. J. Shellfish Res. 30 (3), 659–669. https://doi.org/10.2983/035.030.0308.
Waldbusser, G.G., Voigt, E.P., Bergschneider, H., Green, M.A., Newell, R.I.E., 2011b. Biocalcification in the Eastern oyster (*Crassostrea virginica*) in relation to long-term trends in Chesapeake Bay pH. Estuar. Coast 34 (2), 221–231. https://doi.org/10.1007/s12237-010-9307-0.
Waldbusser, G.G., Hales, B., Langdon, C.J., Haley, B.A., Schrader, P., Brunner, E.L., et al., 2015. Ocean acidification has multiple modes of action on bivalve larvae. PloS One 10 (6), e0128376. https://doi.org/10.1371/journal.pone.0128376.
Waldbusser, G.G., Gray, M.W., Hales, B., Langdon, C.J., Haley, B.A., Gimenez, I., et al., 2016. Slow shell building, a possible trait for resistance to the effects of acute ocean acidification. Limnol. Oceanogr. https://doi.org/10.1002/lno.10348.
Walne, P., 1958. Growth of oysters (*Ostrea edulis* L.). J. Mar. Biol. Assoc. U. K. 37 (03), 591–602.
Wang, Q., Cao, R., Ning, X., You, L., Mu, C., Wang, C., et al., 2016. Effects of ocean acidification on immune responses of the Pacific oyster *Crassostrea gigas*. Fish Shellfish Immunol. 49, 24–33. https://doi.org/10.1016/j.fsi.2015.12.025.
Weatherdon, L.V., Magnan, A.K., Rogers, A.D., Sumaila, U.R., Cheung, W.W.L., 2016. Observed and projected impacts of climate change on marine fisheries, aquaculture, coastal tourism, and human health: an Update. Front. Mar. Sci. 3. https://doi.org/10.3389/fmars.2016.00048.
Weiss, R.F., 1970. The solubility of nitrogen, oxygen and argon in water and seawater. Deep-Sea Res. 17, 721–735.
Welladsen, H.M., Southgate, P.C., Heimann, K., 2010. The effects of exposure to near-future levels of ocean acidification on shell characteristics of *Pinctada fucata* (Bivalvia: Pteriidae). Molluscan Res. 30 (3), 125.
Woolmer, A.P., Syvret, M., F, A., 2011. No: 960 P. Restoration of Native Oyster, Ostrea edulis, in South Wales: Options and Approaches. CCW Contract Science Report. pp. 960.
Wright, J.M., Parker, L.M., O'Connor, W.A., Williams, M., Kube, P., Ross, P.M., 2014. Populations of Pacific oysters *Crassostrea gigas* respond variably to elevated CO_2 and predation by *Morula marginalba*. Biol. Bull. 226 (3), 269–281.
Zuur, A.F., Ieno, E.N., Walker, N., Saveliev, A.A., Smith, G.M., 2009. Mixed Effects Models and Extensions in Ecology with R. Springer, New York.
Zwerschke, N., Emmerson, M.C., Roberts, D., O'Connor, N.E., 2016. Benthic assemblages associated with native and non-native oysters are similar. Mar. Pollut. Bull. 111 (1–2), 305–310. https://doi.org/10.1016/j.marpolbul.2016.06.094.
Zwerschke, N., Kochmann, J., Ashton, E.C., Crowe, T.P., Roberts, D., O'Connor, N.E., 2017. Co-occurrence of native *Ostrea edulis* and non-native *Crassostrea gigas* revealed by monitoring of intertidal oyster populations. J. Mar. Biol. Assoc. U. K. 1–10. https://doi.org/10.1017/S0025315417001448. Epub 08/23.